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U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

REN-13087

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

10/089136

INTERNATIONAL APPLICATION NO.

PCT/EP00/09569

INTERNATIONAL FILING DATE

29 September 2000

PRIORITY DATE CLAIMED

1 October 1999

TITLE OF INVENTION

Device For The Carrying Out Of Chemical Or Biological Reactions

APPLICANT(S) FOR DO/EO/US

Wolfgang Heimberg, Markus Schurf, Thomas Herrmann, Matthias Knulle, Tilmann Wagner

Applicant herewith submits to the United States Designated/Elected Office (DO/E O/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☒ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). (Amendment to specification and claims)

Items 11 to 16 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

U.S. APPLICATION NO. (if known, see 37 CFR 1.53) 10/089136		INTERNATIONAL APPLICATION NO. PCT/EP00/09569		ATTORNEY'S DOCKET NUMBER REN-13087	
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17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) : Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$710.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>				CALCULATIONS PTO USE ONLY 	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	32 - 20 =	12	X \$18.00	\$ 216.00	
Independent claims	1 - 3 =	0	X \$80.00	\$ 0.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ \$270.00	\$
TOTAL OF ABOVE CALCULATIONS =				\$ 1,106.00	
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				\$	
SUBTOTAL =				\$ 553.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$ 553.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$	
TOTAL FEES ENCLOSED =				\$ 553.00	
				Amount to be refunded:	\$
				charged:	\$

a. ☒ A check in the amount of \$ 553.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees.
A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
overpayment to Deposit Account No. 180160. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

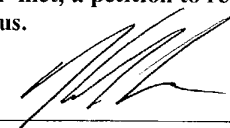
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Cleveland, Ohio 44115-1405


 SIGNATURE:
 Kenneth A. Clark
 NAME
 32,119
 REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#3/a

In re application of	:	
	:	Group Art Unit
Wolfgang Heimberg et al.	:	
	:	Examiner
Serial No.	:	
	:	Confirmation No.
Filed: March 25, 2002	:	
	:	U.S. National Filing of
For: DEVICE FOR THE CARRYING OUT OF	:	PCT/EP00/09569
CHEMICAL OR BIOLOGICAL REACTIONS:	:	Filed 29 September 2000

Box PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Dear Sir:

Prior to calculating the filing fee and examining the above noted application, please proceed to amend the application as follows:

In the Claims:

Please cancel pending claims 1-18 without prejudice.

Please add new claims 19-50 as follows:

19. Device for the carrying out of chemical or biological reactions with a reaction vessel receiving element for receiving a microtiter plate with several reaction vessels, wherein the reaction vessel receiving element has several recesses arranged in a regular pattern to receive the respective reaction vessels, a heating device for heating the reaction vessel receiving element, and a cooling device for cooling the reaction vessel receiving element, wherein a) the reaction vessel receiving element is divided into several segments, b) each segment is assigned a heating device, wherein the

heating devices may be actuated independently of one another, c) the individual segments are thermally decoupled in such a way that different temperature levels may be set and maintained in two adjacent segments.

20. Device according to claim 19, wherein each segment of the reaction vessel receiving element is assigned a cooling device, wherein the cooling devices may be actuated independently of one another.

21. Device according to claim 19, wherein the segments of the reaction vessel receiving element are each comprised of a base plate with one or more tubular, thin-walled reaction vessel holders, which form one piece together with the base plate.

22. Device according to claim 20, wherein the segments of the reaction vessel receiving element are each comprised of a base plate with one or more tubular, thin-walled reaction vessel holders, which form one piece together with the base plate.

23. Device according to claim 19, wherein the individual segments are thermally decoupled by means of an air gap formed between adjacent segments.

24. Device according to claim 22, wherein the individual segments are thermally decoupled by means of an air gap formed between adjacent segments.

25. Device according to claim 19, wherein the individual segments are thermally decoupled by means of a gap, formed between adjacent segments, in which a thermal insulator is inserted.

26. Device according to claim 24, wherein the individual segments are thermally decoupled by means of a gap, formed between adjacent segments, in which a thermal insulator is inserted.

27. Device according to claim 19, wherein each of the heating devices has a Peltier element, wherein each case one segment of the reaction vessel receiving element is assigned a Peltier element, and the Peltier elements are thermally coupled to the respective segments.

28. Device according to claim 26, wherein each of the heating devices has a Peltier element, wherein in each case one segment of the reaction vessel receiving element is assigned a Peltier element, and the Peltier elements are thermally coupled to the respective segments.

29. Device according to claim 19, wherein the cooling devices comprise a Peltier element and/or a heat exchanger, wherein in each case one segment of the reaction vessel receiving element is assigned a Peltier element and/or a heat exchanger.

30. Device according to claim 29, wherein the cooling devices comprise a Peltier element and/or a heat exchanger, wherein in each case one segment of the reaction vessel receiving element is assigned a Peltier element and/or heat exchanger.

31. Device according to claim 29, wherein the heat exchanger is provided with cooling ducts through which a fluid may flow, wherein the fluidic flow of individual heat exchangers may be controlled independently of one another.

32. Device according to claim 30, wherein the heat exchanger is provided with cooling ducts through which a fluid may flow, wherein the fluidic flow of individual heat exchangers may be controlled independently of one another.

33. Device according to claim 31, wherein that the fluid is a cooling fluid, in particular water.

34. Device according to claim 32, wherein that the fluid is a cooling fluid, in particular water.

35. Device according to claim 19, wherein the reaction vessel receiving element is divided into at least four segments.

36. Device according to claim 34, wherein the reaction vessel receiving element is divided into at least four segments.

37. Device according to claim 19, wherein the individual segments each have the same number of recesses.

38. Device according to claim 36, wherein the individual segments each have the same number of recesses.

39. Device according to claim 19, wherein on their side edges the segments have downwards-facing hook elements by which they rest on ties.

40. Device according to claim 38, wherein on their side edges the segments have downwards-facing hook elements by which they rest on ties.

41. Device according to claim 19, wherein each segment is assigned a temperature sensor with which the temperature of the segment concerned is sensed, with the temperature of the segment being controlled on the basis of the temperatures sensed by the individual sensors.

42. Device according to claim 40, wherein each segment is assigned a temperature sensor with which the temperature of the segment concerned is sensed, with the temperature of the segment being controlled on the basis of the temperatures sensed by the individual sensors.

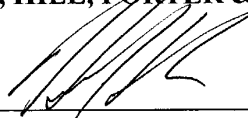
43. Device according to claim 19, wherein each segment is assigned one or more temperature equalisation elements.

44. Device according to claim 42, wherein each segment is assigned one or more temperature equalisation elements.

50. Device according to claim 48, wherein in one operating mode the segments are so actuated that the temperature difference between adjacent segments is less than a predetermined temperature difference (ΔT).

Respectfully submitted,

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Device for the carrying out of chemical or biological reactions

The present invention relates to a device for the carrying out of chemical or biological reactions with

10 a reaction vessel receiving element for receiving reaction vessels, wherein the reaction vessel receiving element has several recesses arranged in a regular pattern to receive reaction vessels, a heating device for heating the reaction vessel receiving element, and a cooling device for cooling the reaction vessel receiving element.

15 Such devices are described as thermocyclers or thermocycling devices and are used to generate specific temperature cycles, i.e. to set predetermined temperatures in the reaction vessels and to maintain predetermined intervals of time.

20 A device of this kind is known from US 5,525,300. This device has four reaction vessel receiving elements, each with recesses arranged in a regular pattern. The pattern of the recesses corresponds to a known pattern of reaction vessels of standard microtiter plates, so that microtiter plates with their reaction vessels may be inserted in the recesses.

25

The heating and cooling devices of a reaction vessel receiving element are so designed that a temperature gradient extending over the reaction vessel receiving element may be generated. This means that, during a temperature cycle, different temperatures may be obtained in the individual reaction vessels.

30 This makes it possible to carry out certain experiments at different temperatures simultaneously.

This temperature gradient is used to determine the optimal denaturing temperature, the optimal annealing temperature and the optimal elongation temperature of a PCR reaction. To achieve this, the same reaction mixture is
5 poured into the individual reaction vessels, and the temperature cycles necessary to perform the PCR reaction are executed. Such a temperature cycle comprises the heating of the reaction mixture to the denaturing temperature, which usually lies in the range 90°-95°C, cooling to the annealing temperature, which is usually in the range 40°-60°C, and heating to the elongation temperature, which is
10 usually in the range 70-75°C. A cycle of this kind is repeated several times, leading to amplification of a predetermined DNA sequence.

Since a temperature gradient can be set, different but predetermined temperatures are set in the individual reaction vessels. After completion of the
15 cycles it is possible to determine, with the aid of the reaction products, those temperatures at which the PCR reaction will give the user the optimal result. Here the result may be optimised e.g. in respect of product volume or also product quality.

20 The annealing temperature, at which the primer is added, has a powerful influence on the result. However the elongation temperature too can have beneficial or adverse effects on the result. At a higher elongation temperature, the addition of the bases is accelerated, with the probability of errors increasing with higher temperature. In addition, the life of the polymerase is shorter at a
25 higher elongation temperature.

A thermocycling device, by which the temperature gradient may be set, makes it much easier to determine the desired temperatures, since a reaction mixture may simultaneously undergo cycles at different temperatures in a single thermocycling
30 device.

To determine the optimal residence times and rates of temperature change it is necessary to have either several thermocycling devices or a multiblock thermocycling device, or to carry out tests in several consecutive test series. The acquisition of several thermocycling devices or of a multiblock thermocycling device is costly and the carrying-out of several consecutive test series takes too long. In addition, handling is laborious when only part of the reaction vessels of several microtiter plates is filled, with each of the latter being tested and optimised in separate test series. This is especially disadvantageous in the case of device which operate automatically and in which the reaction mixtures are subject to further operations, since several microtiter plates must then be handled separately. It is also extremely impractical when only part of the reaction vessels of the microtiter plates is filled, since the devices for further processing, such as e.g. sample combs for transferring the reaction products to an electrophoresis apparatus, are often laid out on the grid of the microtiter plates, which means that further processing is correspondingly limited if only part of the reaction vessels of the microtiter plate is used.

PCT/EP 00/09569

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US 5,819,842 discloses a device for the individual, controlled heating of several samples. This device has several flat heating elements arranged in a grid pattern on a work surface. Formed below the heating elements is a cooling device which extends over all the heating elements. In operation a specially designed sample plate is placed on the work surface. This sample plate has a grid plate, covered on the underside by a film. The samples are poured into the recesses of the grid plate. In this device the samples lie on the individual heating elements, separated from them only by the film. By this means, direct heat transfer is obtained. The drawback of this device, however, is that no commonly available microtiter plate can be used.

With increasing automation in biotechnology, thermocyclers are increasingly being used in automated production lines and with robots as one of several work stations. Here it is customary for the samples to be passed in microtiter plates from one work station to the next. If the device according to US 5,819,842 were to be used in such an automated production process, it would be necessary for the samples to be pipetted out of a microtiter plate into the specially designed sample plate before temperature adjustment, and from the sample plate into a microtiter plate after temperature adjustment. Here there is a risk of contamination of the samples. The use of this specially designed sample plate must therefore be regarded as extremely disadvantageous.

The invention is based on the problem of developing the device described above in such a way that the disadvantages described above are avoided and the parameters of the PCR process may be optimised with great flexibility.

To solve this problem the invention has the features specified in claim 1. Advantageous developments thereof are set out in the additional claims.

The invention is characterised by the fact that the reaction vessel receiving element is divided into several segments, with the individual segments thermally

decoupled and each segment assigned a heating device which may be actuated independently.

By this means the individual segments of the device may be set to different
5 temperatures independently of one another. This makes it possible not only to set
different temperature levels in the segments, but also for them to be held for
varying lengths of time or altered at different rates of change. The device
according to the invention thus permits optimisation of all physical parameters
critical for a PCR process, while the optimisation process may be carried out on a
10 single reaction vessel receiving element in which a microtiter plate may be
inserted.

With the device according to the invention it is therefore also possible to optimise
the residence times and rates of temperature change without having to distribute
15 the reaction mixture over different microtiter plates for this purpose.

The thermocycling device according to the invention is in particular suitable for
optimising the multiplex PCR process, in which several different primers are
used.

20

The above problem, and the features and advantages according to the present
invention, may be better understood from the following detailed description of
preferred embodiments of the present invention and with reference to the
associated drawings.

25

The invention is explained in detail below with the aid of the drawings. These
show in:

Fig. 1 a section through a device according to the invention for carrying
30 out chemical or biological reactions in accordance with a first
embodiment,

Fig. 2 a section through an area of a device according to the invention for carrying out chemical or biological reactions in accordance with a second embodiment,

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Fig. 3 a schematic plan view of the device of Fig. 2,

Fig. 4 a schematic plan view of a device according to a third embodiment,

10 Fig. 5 an area of the device of Fig. 4 in a sectional view along the line A-A,

Figs. 6 to 9 schematic plan views of reaction vessel receiving elements with differing segmentation

15

Fig. 10 a clamping frame in plan view

Fig. 11 a device according to the invention in which segments of a reaction vessel receiving element are fixed by the clamping frame according to Fig. 10, and

20

Fig. 12 a further embodiment of a device according to the invention in section, in which segments of a reaction vessel receiving element are fixed by the clamping frame according to Fig. 10.

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Fig. 1 shows a first embodiment of the device 1 according to the invention for carrying out chemical or biological reactions in a schematic sectional view.

The device has a housing 2 with a bottom 3 and side walls 4. Located just above and parallel to the bottom 3 is an intermediate wall 5, on which are formed

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PCT/EP 00/09569

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several bases 5a. In the embodiment shown in Fig. 1, a total of six bases 5a are provided, arranged in two rows of three bases 5a each.

Mounted on each of the bases 5a is a heat exchanger 6, a Peltier element 7 and a segment 8 of a reaction vessel receiving element 9. The heat exchanger 6 is part of a cooling device and the Peltier element 7 is part of a combined heating and cooling device. The elements (heat exchanger, Peltier element, segment) mounted on the bases 5a are bonded by an adhesive resin with good heat conducting properties, so that good heat transfer is realised between these elements, and the elements are also firmly connected to a segment element 10. the device has altogether six such segment elements 10. Instead of adhesive resin, a heat conducting film or a heat conducting paste may also be provided.

Each of the segments 8 of the reaction vessel receiving element 9 has a base plate 11 on which tubular, thin-walled reaction vessel holders 12 are integrally formed. In the embodiment depicted in Fig. 1, in each case 4 x 4 reaction vessel holders 12 are arranged on a base plate 11. The distance d between adjacent segments 8 is such that the reaction vessel holders 12 of all segments 8 are arranged in a regular pattern with constant grid spacing D. The grid spacing D is chosen so that a standardised microtiter plate with its reaction vessels may be inserted in the reaction vessel holders 12.

By providing the distance d between adjacent segments, an air gap which thermally decouples the segments 8 and segment elements 10 respectively is formed.

The reaction vessel holders 12 of the device shown in Fig. 1 form a grid with a total of 96 reaction vessel holders, arranged in eight rows each with twelve reaction vessel holders 12.

The Peltier elements 7 are each connected electrically to a first control unit 13. Each of the heat exchangers 6 is connected via a separate cooling circuit 14 to a second control unit 15. The cooling medium used is for example water, which is cooled in the cool temperature control unit before transfer to one of the heat exchangers 6.

The first control unit 13 and the second control unit 15 are connected to a central control unit 16 which controls the temperature cycles to be implemented in the device. Inserted in each cooling circuit 14 is a control valve 19, which is controlled by the central control unit 16 to open or close the respective cooling circuit 14.

Pivotably attached to the housing 2 is a cover 17 in which additional heating elements 18 in the form of Peltier elements, heating films or semiconductor heating elements may be located. The heating elements 18 form cover heating elements, each assigned to a segment 8 and separately connected to the first control unit 13, so that each heating element 18 may be individually actuated.

The mode of operation of the device according to the invention is explained in detail below.

There are three modes of operation.

In the first operating mode all segments are set to the same temperature, i.e. the same temperature cycles are run on all segments. This operating mode corresponds to the operation of a conventional thermocycling device.

In the second operating mode the segments are actuated with different temperatures, wherein the temperatures are so controlled that the temperature difference ΔT of adjacent segments 8 is less than a predetermined value K which amounts for example to 5°-15°C. The value to be chosen for K depends on the

quality of the thermal decoupling. The better the thermal decoupling, the greater the value which can be chosen for K.

5 The temperature cycles input by the user may be distributed automatically by the central control unit 16 to the segments 8, so that the temperature differences between adjacent segments are kept as small as possible.

10 This second operating mode may be provided with a function by which the user inputs only a single temperature cycle or PCR cycle, and the central control unit 16 then varies this cycle automatically. The parameters to be varied, such as temperature, residence time or rate of temperature change, may be chosen by the user separately or in combination. Variation of the parameters is effected either by linear or sigmoidal distribution.

15 In the third operating mode, only part of the segments is actuated. In plan view (Fig. 3, Fig. 4, Figs. 6 to 9) the segments 8 have side edges 20. In this operating mode, the segments 8 adjacent to the side edges of an actuated segment 8 are not actuated. If the segments 8 themselves form a regular pattern (Fig. 3, Fig. 4, Fig. 6, Fig. 7 and Fig. 8), then the actuated segments are distributed in a
20 chessboard pattern. In the embodiments shown in Figs. 1 to 4, three of the six segments 8 can be actuated, namely the two outer segments of one row and the middle segment of the other row.

25 In this operating mode the actuated segments are not influenced by the other segments, and their temperature may be set completely independently of the other actuated segments. By this means it is possible to run quite different temperature cycles on the individual segments, with one of the segments for example heated up to the denaturing temperature and another held at the annealing temperature. Thus it is possible for the residence times, i.e. the
30 intervals of time for which the denaturing temperature, the annealing temperature and the elongation temperature are held, also the rates of temperature change,

to be set as desired, and run simultaneously on the individual segments. In this way it is possible to optimise not only the temperatures, but also the residence times and the rates of temperature change.

5 In this operating mode it may be expedient to heat the non-actuated segments 8 a little, so that their temperature lies roughly in the range of the lowest temperature of the adjacent actuated segments. This avoids the non-actuated segments forming a heat sink for the actuated segments and affecting their temperature profile adversely.

10

A second embodiment of the device according to the invention is shown in Figs. 2 and 3. the basic design corresponds to that of Fig. 1, so that identical parts have been given the same reference number.

15 The second embodiment differs from the first embodiment by virtue of the fact that the side edges 20 of the segments 8 adjacent to the side walls 4 of the housing 2 engage in a slot 21 running round the inner face of the side walls 4, and are fixed therein for example by bonding. By this means the individual segment elements 10 are fixed in space, thereby ensuring that despite the form
20 of the gaps between the segment elements 10, all reaction vessel holders 12 are arranged in the pattern of the reaction vessels of a microtiter plate. The side walls 4 of the housing 2 are made of a non heat conducting material. This embodiment may also be modified such that the slot 21 is introduced in a frame formed separately from the housing 2. The frame and the segments inserted in it form a
25 part which may be handled separately during production and is bonded to the heating and cooling devices.

A third embodiment is shown schematically in Figs. 4 and 5. In this embodiment, ties 22 of non heat conducting material are located somewhat below the base
30 plates 11 of the segments 8 in the areas between the segment elements 10 and between the segment elements 10 and the side walls 4 of the housing 2. On the

side edges 20 of the segments 8 and of the base plates 11 respectively are formed hook elements 23 which are bent downwards. These hook elements 23 engage in corresponding recesses of the ties 22 (Fig. 5), thereby fixing the segments 8 in their position. The hook elements 23 of adjacent segments 8 are offset relative to one another. The ties 22 thus form a grid, into each of the openings of which a segment 8 may be inserted.

This type of position fixing is very advantageous since the boundary areas between the segments 8 and the ties 22 are very small, so that heat transfer via the ties 22 is correspondingly low. Moreover this arrangement is easy to realise even in the confined space conditions between adjacent segment elements.

Shown in schematic plan view in Figs. 6 to 9 are reaction vessel receiving elements 9 which represent further modifications of the device according to the invention. In these reaction vessel receiving elements 9, the individual segments 8 are joined by webs 24 of a thermally insulating material joined to form a single unit. The ties 22 are arranged between the side edges 20 of the base plates 11, to which they are fixed for example by bonding.

The segmentation of the reaction vessel receiving element of Fig. 6 corresponds to that of the first and second embodiment (Fig. 1-3), in which 4 x 4 reaction vessel holders are arranged on each segment 8.

The reaction vessel receiving element 9 shown in Fig. 7 is comprised of 24 segments 8 each with 4 x 4 reaction vessel holders 12, while the segments 8 are in turn connected by means of thermally insulating webs 24.

In the reaction vessel receiving element 9 shown in Fig. 8, each segment 8 has only a single reaction vessel holder 12.

For the relatively finely sub-divided reaction vessel receiving elements 9 it is expedient to integrate temperature sensors in the thermocycling device. These temperature sensors sense the temperatures of the individual segments, so that the temperature of the segments 8 is regulated in a closed control loop on the basis of the temperature values determined by the temperature sensors.

Infrared sensors may for example be used as temperature sensors, located e.g. in the cover. With this sensor arrangement it is possible to sense the temperature of the reaction mixture directly.

Fig. 9 shows a reaction vessel receiving element 9 with six segments 8, rectangular in plan view, and a segment 8a in the form of a double cross formed by three intersecting rows of reaction vessel holders 12. The six rectangular segments 8 are each separated from the next rectangular segment by a row or column of reaction vessel holders. This segmentation is especially advantageous for the third operating mode described above, since the rectangular segments 8 are not in contact with one another and may therefore be actuated simultaneously as desired, with only the segment 8a in the form of a double cross not being actuated.

The segments 8 of the reaction vessel receiving element 9 are made from a metal with good heat conducting properties, e.g. aluminium. The materials described above as non-heat conducting materials or thermally insulating materials are either plastics or ceramics.

A further embodiment of the device according to the invention is shown in Fig. 11. In this embodiment the individual segments 8b of the reaction vessel receiving element 9 are fixed in position by means of a clamping frame 25 (Fig. 10).

The clamping frame 25 is grid-shaped and formed by longitudinal ties 26 and cross ties, wherein the ties 26, 27 span openings. Through these openings extend the reaction vessel holders 12 of the segments 8b. In the present embodiment, the ties 26, 27 are for instance in positive contact with the reaction vessel holders 12 and with the base plate 11 which protrudes from the reaction vessel holders. The 25 is provided with holes 28, through which pass bolts 29 for fixing the clamping frame to a thermocycling device 1.

Located below each of the segments 8b is a separately actuatable Peltier element 7 and a cooling element 30 which extends over the area of all the segments 8b. Located in each case between the cooling element 30 and the Peltier element 7, and between the Peltier element 7 and the respective segment 8b is a heat conducting foil 31. The cooling element 30 is provided with holes through which extend the bolts 29, each fixed by a nut 32 to the side of the cooling element 30 facing away from the reaction vessel receiving element 9.

The clamping frame 25 is made from a non heat conducting material, in particular POM or polycarbonate. It therefore allows a fixing of the segments 8b of the reaction vessel receiving element 9 wherein the individual elements between the segments 8b and the cooling element 30 are under tension, thereby ensuring good heat transfer in the vertical direction between the individual elements. Since the clamping frame itself has poor heat conducting properties, the heat transfer between two adjacent segments 8b is kept low. For further reduction of heat transfer between two adjacent segments, the surfaces of the clamping frame 25 in contact with the segments 8b may be provided with narrow webs, so that in the areas adjoining the webs, air gaps are formed between the clamping frame 25 and the segments 8b.

In the embodiment shown in Fig. 11, a so-called heat pipe 33 is fitted between every two rows of reaction vessel holders 12. Such a heat pipe is distributed for example by the company THERMACORE INTERNATIONAL, Inc., USA. It is

comprised of a gastight jacket, in which there is only a small amount of fluid. The pressure in the heat pipe is so low that the fluid is in a state of equilibrium between the liquid and the gaseous aggregate state, and consequently evaporates at a warmer section of the heat pipe and condenses at a cooler section. By this means, the temperature between the individual sections is equalised. The fluid used is, for example, water or freon.

Through integration of such a heat pipe in the segments 8b of the reaction vessel receiving element 9, a temperature equalisation is effected over the segment 8b. By this means it is ensured that the same temperature is present over the whole segment 8b.

A further embodiment of the thermocycling device 1 according to the invention is shown in Fig. 12. The design of this thermocycling device 1 is similar to that of Fig. 11, therefore similar parts have been given the same reference numbers.

The segments 8c of this thermocycling device 1, however, have no heat pipe. Instead of heat pipes, a temperature equalisation plate 34 is provided in the area beneath each of the segments 8c. These temperature equalisation plates 34 are flat elements with a surface corresponding to the basic surface of one of the segments 8c. These temperature equalisation plates 34 are hollow bodies with a small amount of fluid, and work on the same principle as the heat pipes. By this means it is once again ensured that there are no temperature variations within a segment 8c.

The temperature equalisation plate may however be made from materials with very good heat conducting properties, such as e.g. copper. Additional heating and/or cooling elements, e.g. heating foils, heating coils or Peltier elements, may be integrated in such a temperature equalisation plate. The heating and cooling elements support homogeneity and permit more rapid heating and/or cooling

rates. A Peltier element, which generally does not have an even temperature distribution, is preferably combined with a flat heating element.

The invention is described above with the aid of embodiments with 96 recesses for receiving a microtiter plate with 96 reaction vessels. The invention is not however limited to this number of recesses. Thus for example the reaction vessel receiving element may also have 384 recesses to receive a corresponding microtiter plate. With regard to features of the invention not explained in detail above, express reference is made to the claims and the drawing.

In the embodiments described above, a cooling device with a fluid cooling medium is used. Within the scope of the invention it is also possible to use a gaseous cooling medium, in particular air cooling, instead of a fluid cooling medium.

The reaction vessel receiving elements described above are comprised of a base plate with roughly tubular reaction vessel holders. Within the scope of the invention it is also possible to use a metal block, in which recesses to receive the reaction vessels of the microtiter plate are made.

List of References

	1	thermocycling device	25	clamping frame
	2	housing	26	longitudinal tie
5	3	bottom	27	cross tie
	4	side wall	28	hole
	5	intermediate wall	29	bolt
	5a	base	30	cooling element
	6	heat exchanger	31	heat conducting foil
10	7	Peltier element	32	nut
	8	segment	33	heat pipe
	8a	segment in the form of a double cross	34	temperature equalisation plate
	9	reaction vessel receiving element		
15	10	segment element		
	11	base plate		
	12	reaction vessel holder		
	13	first control unit		
	14	cooling circuit		
20	15	second control unit		
	16	central control unit		
	17	cover		
	18	heating element		
	19	control valve		
25	20	side edge		
	21	slot		
	22	ties		
	23	hook element		
	24	web		

Patent Claims

1. Device for the carrying out of chemical or biological reactions with
5 a reaction vessel receiving element (9) for receiving a microtiter plate with
several reaction vessels, wherein the reaction vessel receiving element (9) has
several recesses arranged in a regular pattern to receive the respective reaction
vessels,
a heating device (7) for heating the reaction vessel receiving element (9), and
10 a cooling device (6) for cooling the reaction vessel receiving element (9),
characterised in that
the reaction vessel receiving element (9) is divided into several segments (8),
with the individual segments (8) thermally decoupled and each segment (8)
assigned a heating device (7) which may be actuated independently of the
15 others.
2. Device according to claim 1,
characterised in that
each segment (8) of the reaction vessel receiving element (9) is assigned a
20 cooling device (6), wherein the cooling devices (6) may be actuated
independently of one another.
3. Device according to claim 1 or 2,
characterised in that
25 the segments (8) of the reaction vessel receiving element (9) are each comprised
of a base plate (11) with one or more tubular, thin-walled reaction vessel holders
(12), which form one piece together with the base plate (11).
4. Device according to any of claims 1 to 3,
30 characterised in that

the individual segments (8) are thermally decoupled by means of an air gap formed between adjacent segments (8).

5. Device according to any of claims 1 to 3,

5 characterised in that

the individual segments (8) are thermally decoupled by means of a gap, formed between adjacent segments (8), in which a thermal insulator is inserted.

6. Device according to any of claims 1 to 5,

10 characterised in that

each of the heating devices has a Peltier element (7), wherein in each case one segment (8) of the reaction vessel receiving element (9) is assigned a Peltier element (7), and the Peltier elements (7) are thermally coupled to the respective segments (8).

15

7. Device according to any of claims 1 to 6,

characterised in that

the cooling devices comprise a Peltier element (7) and/or a heat exchanger (6), wherein in each case one segment (8) of the reaction vessel receiving element

20

(9) is assigned a Peltier element (7) and/or a heat exchanger (6).

8. Device according to claim 7,

characterised in that

the heat exchanger (6) is provided with cooling ducts through which a fluid may flow, wherein the fluidic flow of individual heat exchangers (6) may be controlled independently of one another.

25

9. Device according to claim 8,

characterised in that

30

that the fluid is a cooling fluid, in particular water.

10. Device according to any of claims 1 to 9,
characterised in that
the reaction vessel receiving element (9) is divided into at least four segments
(8).

5

11. Device according to any of claims 1 to 10,
characterised in that
the individual segments (8) each have the same number of recesses.

10 12. Device according to any of the preceding claims,
characterised in that
on their side edges (20) the segments (8) have downwards-facing hook elements
(23) by which they rest on ties 22.

15 13. Device according to any of claims 1 to 12,
characterised in that
each segment (8) is assigned a temperature sensor with which the temperature
of the segment (8) concerned is sensed, with the temperature of the segment (8)
being controlled on the basis of the temperatures sensed by the individual
20 sensors.

14. Device according to any of claims 1 to 13,
characterised in that
each segment (8b, 8c) is assigned one or more temperature equalisation
25 elements (33, 34).

15. Control unit to actuate the heating device and the cooling device of a device
for the carrying out of chemical or biological reactions, which is designed
according to any of claims 1 to 14,
30 characterised in that

the control unit (13, 16) is so designed that the heating devices (7) of the individual segments (8) may be actuated individually.

16. Control unit according to claim 15,

5 characterised in that

the control unit (15, 16) is so designed that the cooling devices of the individual segments (8) may be actuated individually.

17. Control unit according to claim 15 or 16,

10 characterised in that

in one operating mode the control unit (13, 15, 16) actuates only a part of the segments, wherein the segments (8) have side edges (20), and the segments (8) adjoining the side edges (20) of an actuated segment (8) are not actuated.

15 18. Control unit according to any of claims 15 to 17,

characterised in that

in one operating mode the segments are so actuated that the temperature difference between adjacent segments (8) is less than a predetermined temperature difference (ΔT).

Summary

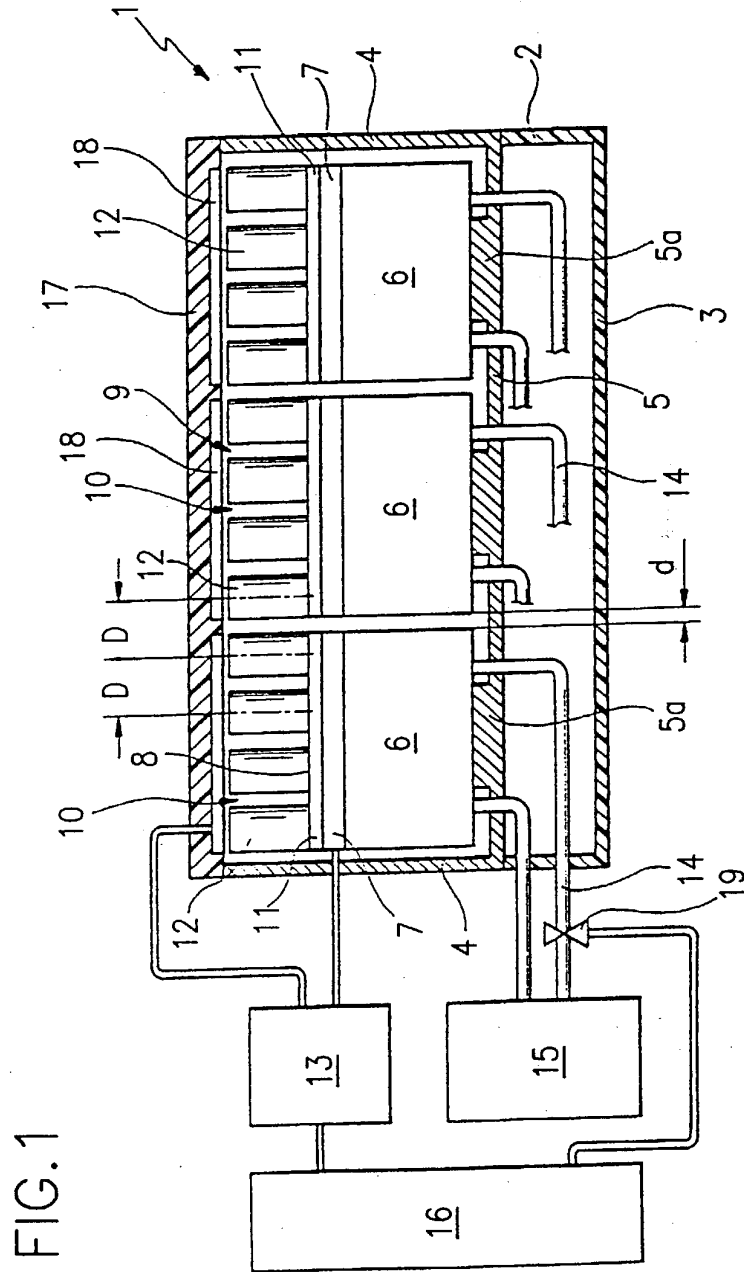
The invention relates to a device for the carrying out of chemical or biological
5 reactions with a reaction vessel receiving element for receiving a microtiter plate
with several reaction vessels, wherein the reaction vessel receiving element has
several recesses arranged in a regular pattern to receive the respective reaction
vessels, a heating device for heating the reaction vessel receiving element, and a
cooling device for cooling the reaction vessel receiving element.

10 The invention is characterised by the fact that the reaction vessel receiving
element is divided into several segments. The individual segments are thermally
decoupled from one another, and each segment is assigned a heating device
which may be actuated independently of the others.

15 By means of the segmentation of the reaction vessel receiving element, it is
possible for zones to be set and held at different temperatures. Since the reaction
vessel receiving element is suitable for receiving standard microtiter plates, the
device according to the invention may be integrated in existing process
20 sequences.

(Fig. 1)

1/7



2/7

FIG.2

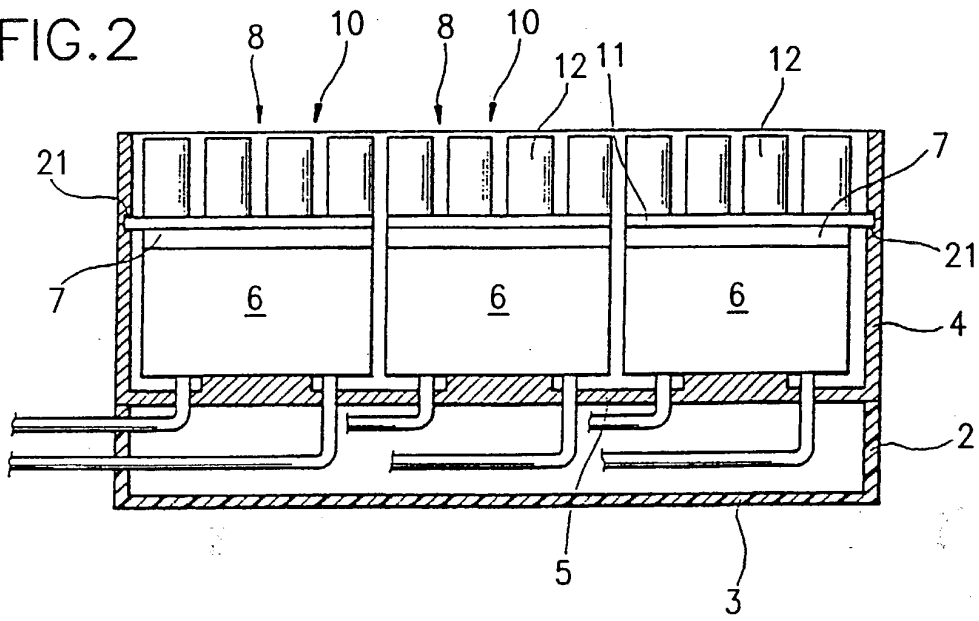
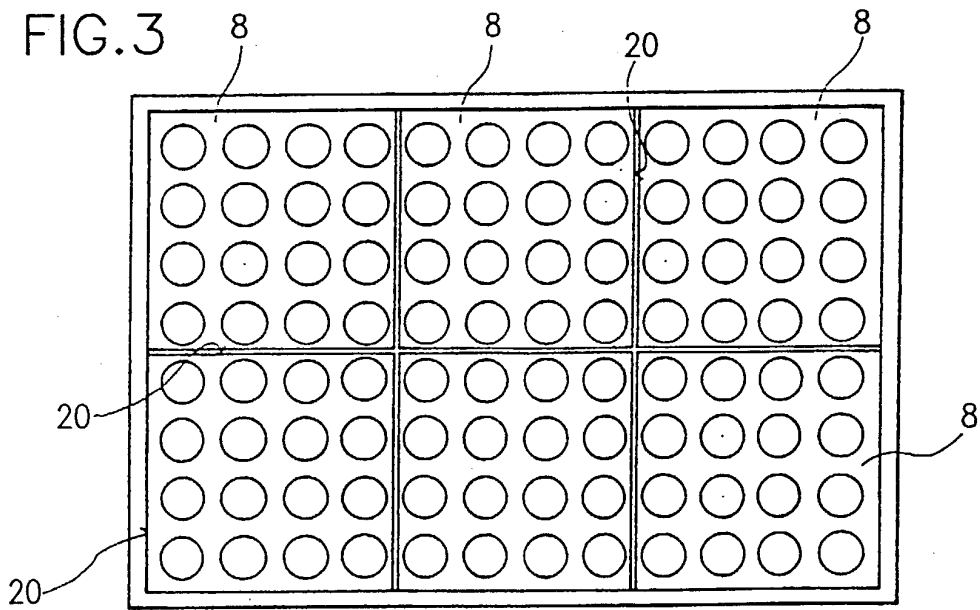


FIG.3



3/7

FIG.4

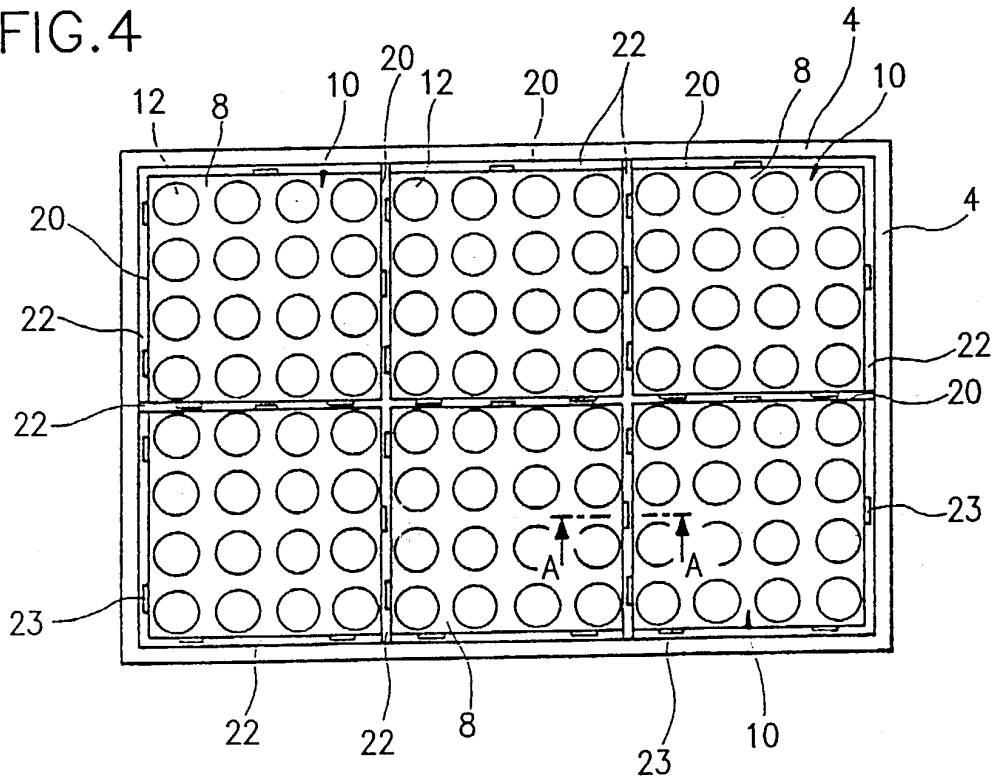
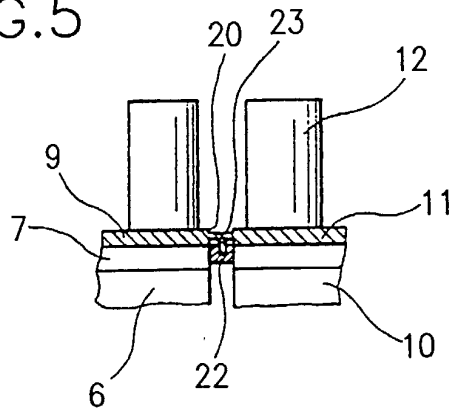


FIG.5



4/7

FIG. 6

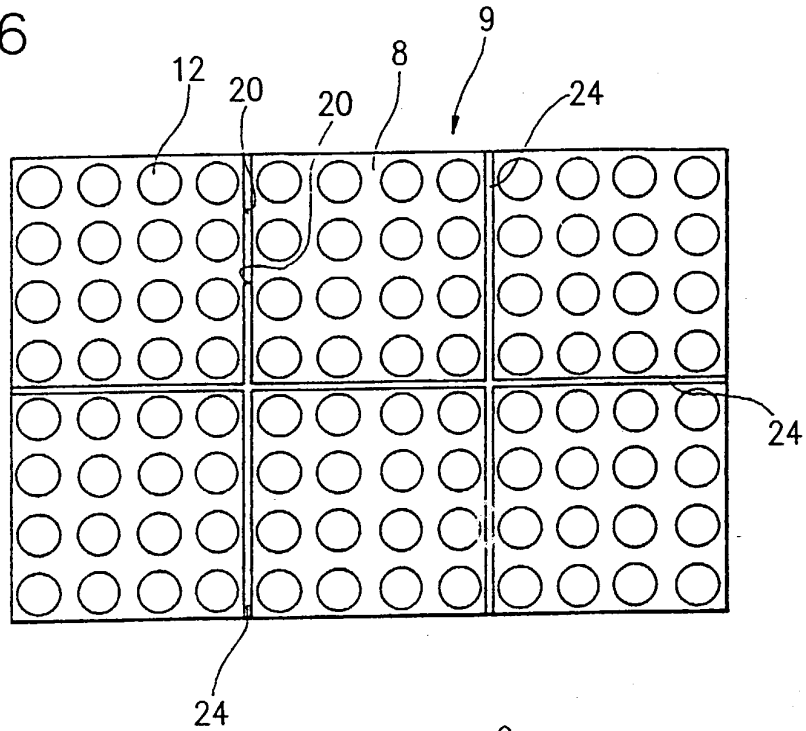
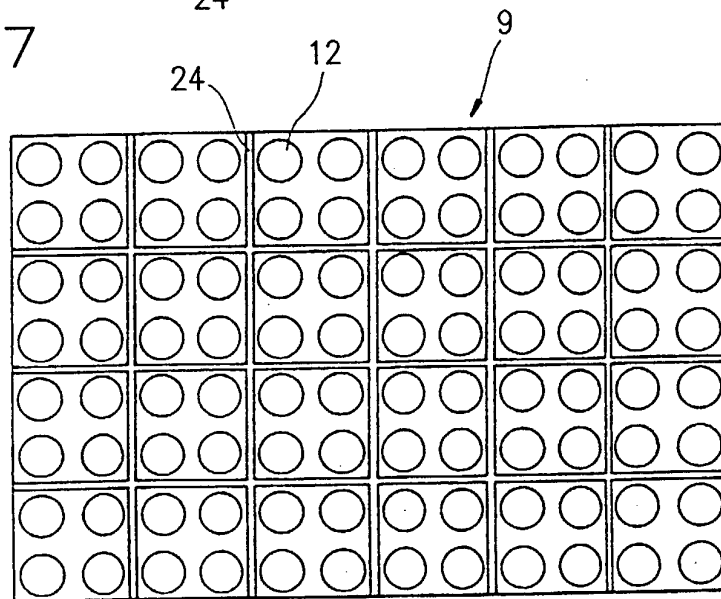


FIG. 7



5/7

FIG. 8

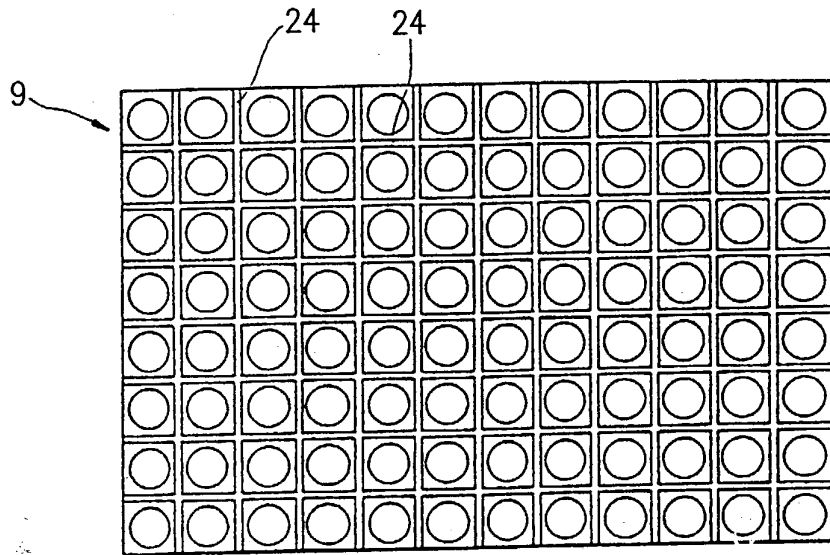
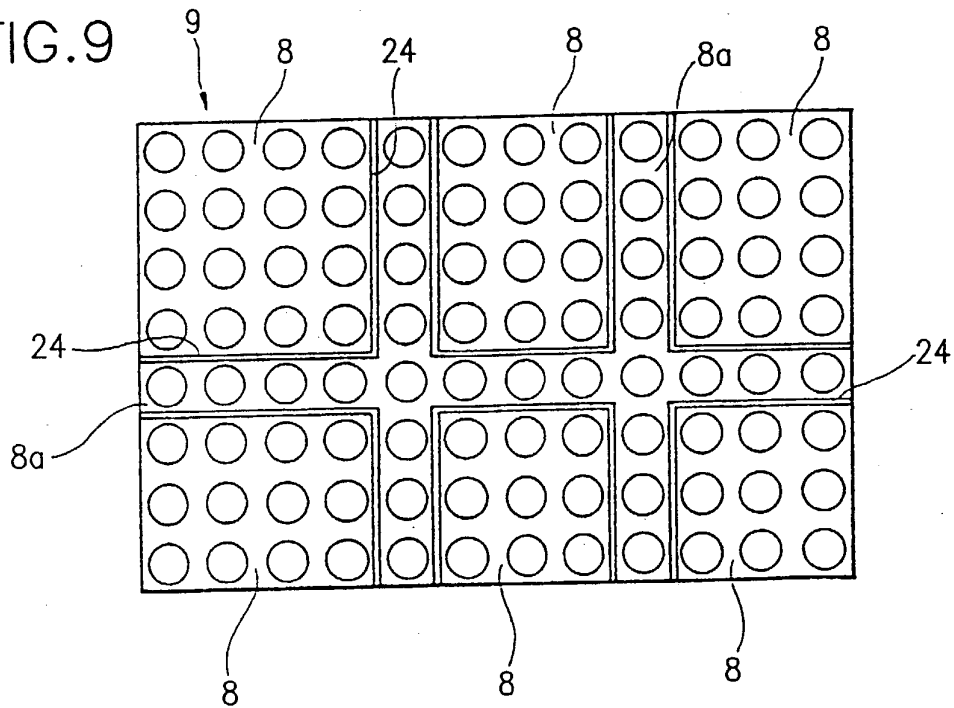
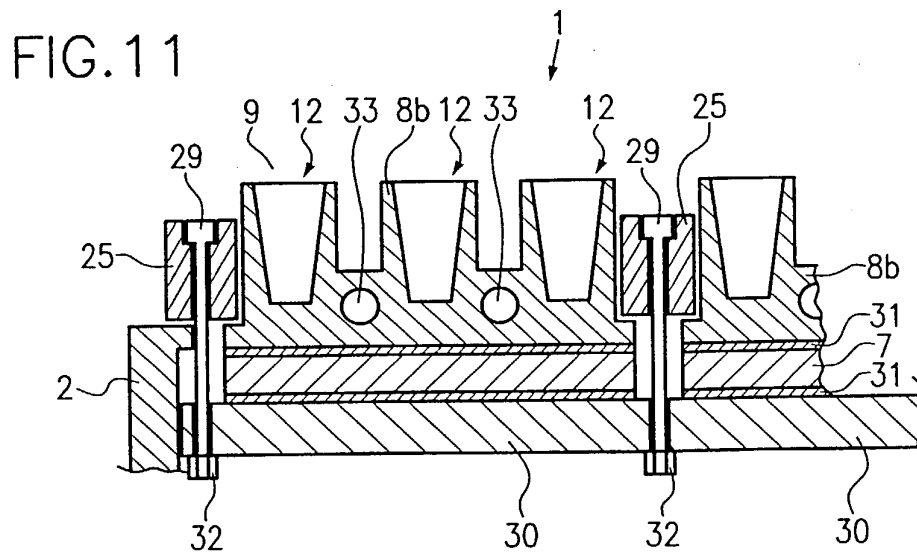


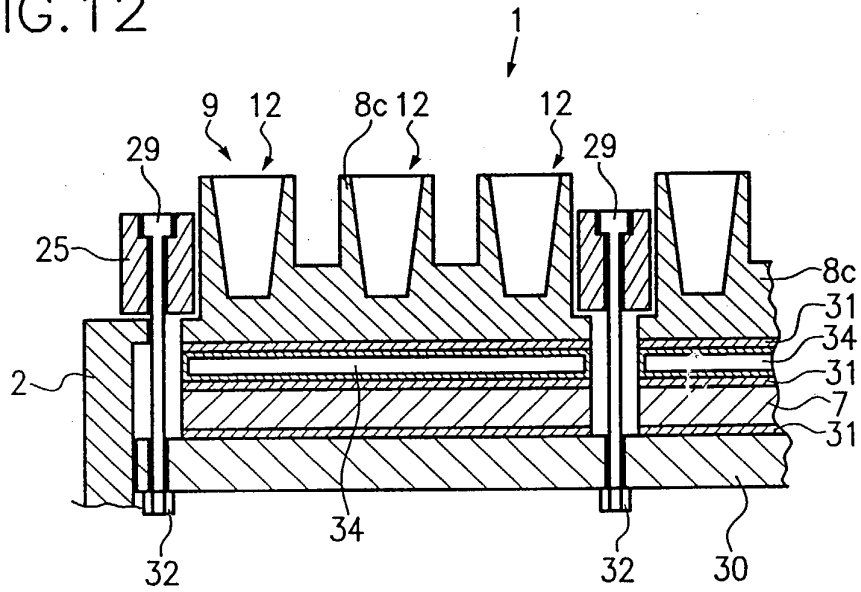
FIG. 9





7/7

FIG.12



#6

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	REN-13087
First Named Inventor	Wolfgang Heimberg
COMPLETE IF KNOWN	
Application Number	10 / 089136
Filing Date	25 March 2002
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Device For The Carrying Out Of Chemical Or Biological Reactions

(Title of the invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 03/25/2002

as United States Application Number or PCT International

Application Number

10/089136

and was amended on (MM/DD/YYYY)

(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
29917313.5 PCT/EP00/09569	Germany Europe	10/01/1999 09/29/2000	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	<input type="checkbox"/> Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

[Page 1 of 2]

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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☒ Additional inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.

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Supplemental Sheet

3-00

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Post Office Address:							
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Post Office Address							
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Post Office Address							
City	Siegsdorf	State		ZIP	83313	Country	German
Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A petition has been filed for this unsigned inventor			
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Tilman				Wagner			
Inventor's Signature	X					Date	X
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